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First straightforward synthesis of 1-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione and structure revision of a bioactive benz[*g*]isochromene-5,10-dione from *Psychotria camponutans*

Jan Jacobs, Sven Claessens, Norbert De Kimpe*

Department of Organic Chemistry, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

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Abstract

For the first time, a synthesis of 1-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**3**), which is claimed to be a bioactive compound isolated from *Psychotria camponutans*, was achieved with a phthalide annulation reaction using 3-cyano-1(3*H*)-isobenzofuranone (**5**) and 5,6-dihydropyran-2-one (**6**) and subsequent reduction of the lactone moiety in the key steps. However, full spectral characterization of the synthesized target compound revealed that the isolated compound is not 1-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**3**). Structure revision shows the previously isolated compound to be the known psychorubrin (**2**).

Keywords: Pyranonaphthoquinones; Psychorubrin; Quinones; *Psychotria camponutans*; *Psychotria rubra*; *Plasmodium falciparum*

1. Introduction

Pyranonaphthoquinone antibiotics have been isolated from higher plants and have attracted considerable synthetic attention as a result of their interesting antimicrobial and antiparasitical properties. For instance, pentalongin (**1**) is the major constituent of *Pentas longiflora* Oliv. (Rubiaceae), a woody herb from Central East Africa (Rwanda, Burundi) also locally known as 'Isagara', and is used in African traditional medicine for its antifungal properties.[1] Psychorubrin (**2**) is a cytotoxic pyranonaphthoquinone, which has been isolated from *Pyschotria rubra* and is known as 'Chiu Chieh Mu' in the Chinese folk medicine.[2] Its 1-hydroxy isomer, 1-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**3**), is proposed to be isolated from the roots and stem of *Psychotria camponutans* (Rubiaceae) as a result of a bioactivity-guided fractionation.[3] This naturally occurring pyranonaphthoquinone showed strong in vitro activity against multi-drug resistant *Plasmodium falciparum* and a high cytotoxicity against human nasopharyngeal KB cells.[3,4] In view of the interesting physiological activities, a synthetic program was directed towards 1-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**3**) (Fig. 1).

Retrosynthetic analysis suggested that 1-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**3**) can be prepared by a phthalide annulation reaction[5] starting from 3-cyano-1(3*H*)-isobenzofuranone (**5**) and 5,6-dihydropyran-2-one (**6**). Protection of the resulting hydroquinone as the corresponding methyl ether should yield 5,10-dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromen-1-one (**4**). Reduction of the annulated δ-lactone to the corresponding hemiacetal followed by oxidative demethylation should then result in the synthesis of the target compound **3** (Fig. 2).

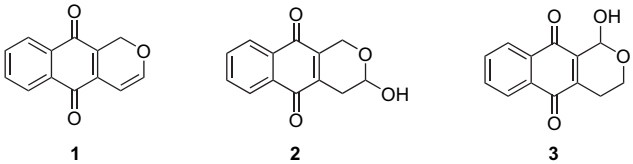

Figure 1.

* Corresponding author. Tel.: +32 9 264 59 51; fax: +32 9 264 62 43.
E-mail address: norbert.dekimpe@ugent.be (N. De Kimpe).

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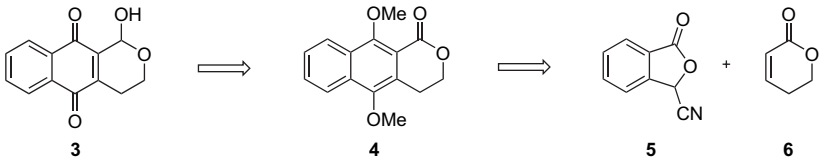
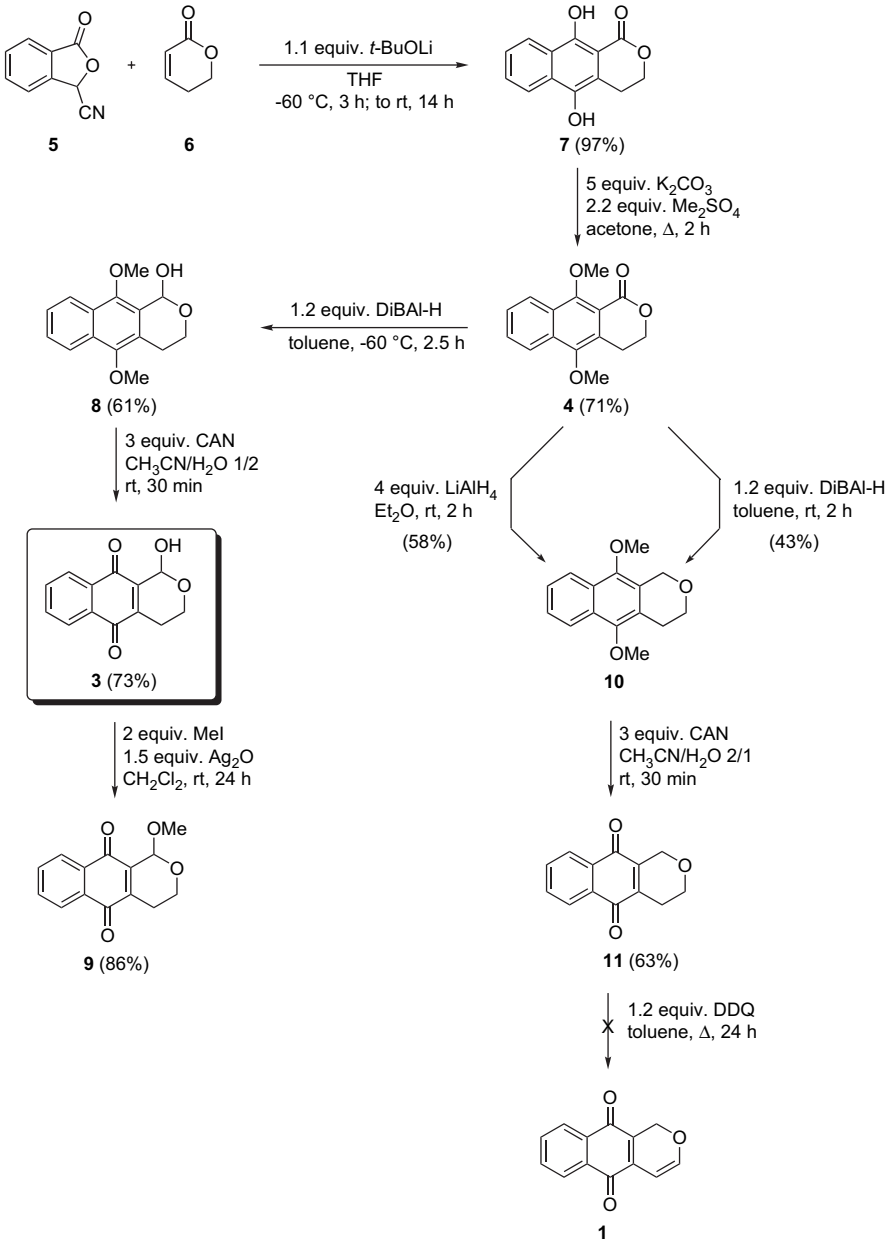


Figure 2.

2. Results and discussion

3-Cyano-1(3*H*)-isobenzofuranone (**5**) was prepared according to literature procedures starting from 2-formylbenzoic acid.[6] A phthalide annulation reaction of this isobenzofuranone with 5,6-dihydropyran-2-one (**6**) using lithium *tert*-butoxide, which was prepared in situ in tetrahydrofuran (THF), resulted in the quantitative formation of 5,10-dihydroxy-3,4-dihydro-benz[*g*]isochromen-1-one (**7**) via Michael-type addition of the carbanion derived from **5** (α to the nitrile) across enone **6** and ring closure of the resulting enolate across the lactone carbonyl (Scheme 1).[5] Since the solubility of this hydroquinone **7** is very limited in most organic solvents and because the hydroquinone moiety oxidizes readily due to oxygen in the

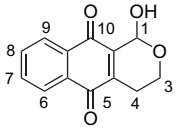


Scheme 1.

air, it was protected as the corresponding double methyl ether **4** upon treatment with dimethyl sulfate and potassium carbonate in refluxing acetone. During the next step, the conversion of the δ-lactone moiety of 5,10-dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromen-1-one (**4**) to the corresponding hemiacetal **8** was accomplished using diisobutyl aluminum hydride (DiBAl-H) in toluene at −60 °C. However, when this reaction was performed at room temperature the δ-lactone moiety was fully reduced upon acidic workup and resulted in the isolation of 5,10-dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromene (**10**) in 43% yield. Since the amount of added DiBAl-H was restrictive in this reaction and the use of lithium aluminum hydride is a well known alternative for the reduction of lactones, an attempt was made to improve the yield for the synthesis of benz[*g*]isochromene **10**. Normally, lactones are reduced to the corresponding diol by lithium aluminum hydride. However, thanks to the directing effect of the methoxy group, one OH-substituent is eliminated and the remaining alcohol gives rise to ring closure on the quinone methide. Accordingly, the use of lithium aluminum hydride in diethyl ether followed by acidic workup resulted in the isolation of 5,10-dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromene **10** in 58% yield. Oxidative demethylation of 5,10-dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromene (**10**) and 5,10-dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromen-1-ol (**8**) using cerium(IV) ammonium nitrate (CAN) in aqueous acetonitrile gave 1-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**3**) and 3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**11**) in 73% and 63% yield, respectively. Treatment of the target compound **3** with methyl iodide and silver(I) oxide in dichloromethane furnished 1-methoxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**9**) in 86% yield. Because of the interest in pentalongin (**1**), a final attempt was made to synthesize this natural product by means of oxidation of 3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**11**) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in boiling toluene. However, this procedure was found to be unsuccessful as the starting material was recovered. Since the synthesis of pentalongin has already been investigated intensively in our department[1d] and was not primarily targeted within this work, it was not investigated further. Although the isolation of 5,10-dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromene (**10**) was the result of an initially undesired side-reaction, it is nevertheless a useful discovery as this reaction can be used as a possible entry to the 3,4-dihydro-1*H*-isochromenedione skeleton, which is the basic skeleton of many important pyranonaphthoquinone antibiotics.

Having all the analytical data of the alleged natural product **3** in hand, a number of principal differences can be noticed with the reported data (Table 1). However, regarding the reported ^{1}H and ^{13}C NMR data by Solis et al.,[3] some considerations need to be made. First, there is an erroneous assignment of the four aromatic protons typical for the 1,2-annulated benzene ring in the ^{1}H and ^{13}C NMR spectra. As the protons at position 6 and 9 of quinone **3** are at the *ortho*-position of a carbonyl moiety, their chemical shift should accordingly be downfield in comparison with the chemical shift of the protons at position 7 and 8 in the ^{1}H NMR spectra. HSQC (Heteronuclear Spin Quantum Coupling) spectra proved that the almost overlapping signals of C-6 and C-9 also need to be interchanged for the signals at C-7 and C-8. In addition, the reported chemical shift of 143 ppm for an aromatic proton seems to be very odd as this results in a difference of about 10 ppm in the chemical shift with the other corresponding aromatic CH. Normally in these types of quinones, signals for C-7 and C-8 as well as for C-6 and C-9 are almost coinciding and the chemical shift of these carbons is generally upfield to 143 ppm (vide infra).[7] Thirdly, although a d×d×d is reported for each of the protons at position 3 and 4 of 1-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**3**), only two coupling constants are reported originating from one geminal

Table 1
Main differences between the synthesized 1-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**3**) and the product isolated from *Psychotria camponutans*

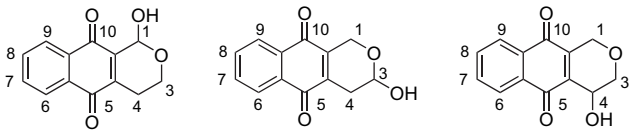


3

^{1}H, ^{13}C NMR δ (ppm)	Compounds	
	Isolated compound[3]	**3**
Solvent	$CDCl_3$/MeOD	$CDCl_3$
H-1	5.30 (1H, t, *J*=3.6 Hz)	6.05 (1H, s)
H-3	4.63–4.84 (2H, ddd, *J*=3.0, 18.5 Hz)	4.01–4.08 and 4.20–4.29 (2H, m)
H-4	2.64–2.84 (2H, ddd, *J*=3.0, 18.9 Hz)	2.66–2.70 (2H, m)
C-1	90.5	85.8
C-3	57.9	56.6
C-4	28.4	22.1
R_f ($CHCl_3$/EtOAc 9:1)	0.42	0.22
UV λ_{max} (MeOH, nm)	211, 245, 261	203, 246, 334
IR ν_{max} (KBr, cm^{-1})	3450, 1660, 1595	3413, 1658, 1635, 1592
EIMS *m/z* (%)[a]	230 (M^+, 10), 212 (70), 201 (15), 184 (100), 173 (15), 156 (35), 128 (85), 115 (24), 104 (32), 76 (45)	229 ($M-H^+$, 85), 227 (11), 211 (9), 201 (42), 199 (30), 186 (100), 185 (93), 173 (15), 171 (30), 158 (14), 159 (9)

[a] Negative mode for compound **3**.

Table 2
Comparison of ^{1}H and ^{13}C NMR spectral data for the natural product isolated from *Psychotria camponutans* with different hydroxy substituted 3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-diones



3 **2** **12**

^{1}H, ^{13}C NMR δ (ppm)	Compounds			
	Isolated compound[3,a]	**3**	**2**[2,9]	**12**[8]
Solvent	$CDCl_3$/MeOD	$CDCl_3$	$CDCl_3$	$CDCl_3$
H-1	5.30 (1H, t)	6.05 (1H, s)	4.70 (1H, dt), 4.80 (1H, dt)	4.49 (1H, dd), 4.76 (1H, dd)
H-3	4.63–4.84 (2H, ddd)	4.01–4.08, 4.20–4.29 (2H, m)	5.49 (1H, t)	3.86 (1H, dd), 4.03 (1H, dd)
H-4	2.64–2.84 (2H, ddd)	2.66–2.70 (2H, m)	2.73 (1H, dq), 2.83 (1H, dq)	4.83 (1H, m)
H-7,8	7.72–7.76 (2H, m)	7.75–7.77 (2H, m)	7.56–7.79 (2H, m)	7.76 (2H, m)
H-6,9	8.03–8.10 (2H, m)	8.09–8.13 (2H, m)	8.03–8.12 (2H, m)	8.09 (2H, m)
C-1	90.5	85.8	57.7	60.2
C-3	57.9	56.6	90.7	69.5
C-4	28.4	22.1	28.0	63.2

[a] Spectral data corrected prior to aforementioned observed mistakes.

and one vicinal coupling.[3] Whether the second geminal coupling constant is 0 Hz or the same as the previously reported geminal coupling constant is not mentioned in the article. Keeping these considerations in mind, there are still some main differences in the analytical data of the synthesized 1-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**3**) and the natural product isolated from *Psychotria camponutans* (Table 1). First of all, chromatographic analysis on silica gel shows that the difference in 'ratio to front'-value is too large to originate from the same compound. Moreover, ^{1}H and ^{13}C NMR spectra show some remarkable differences in chemical shifts for the annelated 2-hydroxy-3,4-dihydropyran moiety, which cannot be entirely ascribed to possible deuterium exchange. Finally, these observations are accompanied by smaller differences in UV, IR, and mass spectral fragmentations.

Since the phthalide annulation reaction is renowned for its regioselectivity,[5] the structure of hydroquinone **7** is secured and is not subjected to any changes of the heterocyclic skeleton in further steps. So despite the aforementioned discrepancy, it is believed that we have indeed the 1-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**3**) in hands. This assumption is justified henceforth thanks to a broad experience related to the synthesis of natural products and especially quinoid compounds in our department. Over the years, numerous pyrananaphthoquinones have been synthesized in our department, including 4-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**12**)[8] and the naturally occurring psychorubrin (**2**).[9] Disposing of the 1-, 3- and 4-hydroxy substituted 3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-diones, a full comparison of the spectral data of these compounds with the spectral data of the natural product isolated from *Psychotria camponutans* forces itself upon us (Table 2).

In this way, the chemical shifts of 4-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**12**) in ^{1}H and ^{13}C NMR immediately exclude this compound to be the natural product isolated from *Psychotria camponutans*. Also, a remarkable resemblance becomes clear between the spectral data of the isolated compound and psychorubrin (**2**). At first sight, only the different multiplicity of the signals in the ^{1}H NMR spectra seem to stand in the way of a possible identification of the isolated natural product with psychorubrin (**2**). However, what appears to be a d×t for CH_aH_b-1 of psychorubrin (**2**) is in fact a d×d×d due to two similar couplings for CH_aH_b-4 of 2.3 and 3.0 Hz, respectively. Therefore, this signal now matches with the reported signal for CH_2-3 of the isolated product (Table 2). The d×q reported for CH_aH_b-4 of psychorubrin (**2**) is in reality a d×d×d×d with 3 similar coupling constants, one vicinal coupling of 3.3 Hz to CH-3 and two couplings of 2.3 Hz to CH_aH_b-1. This information is nevertheless in contrast with the reported d×d×d multiplicity for CH_2-4 of the isolated natural product. However, further

Table 3
Comparison of analytical data of psychorubrin (**2**) with the natural product isolated from *Psychotria camponutans*

	Compounds	
	Isolated natural product[3]	**2**[2,9]
R_f ($CHCl_3$/EtOAc 9:1)	0.42	0.40
UV λ_{max} (MeOH, nm)	211, 245, 261	245, 250, 262, 330
IR ν_{max} (KBr, cm^{-1})	3450, 1660, 1595	3400, 1680, 1590
EIMS *m/z* (%)	230 (M^+, 10), 212 (70), 201 (15), 184 (100), 173 (15), 156 (35), 128 (85), 115 (24), 104 (32), 76 (45)	230 (M^+, 17), 212 (62), 201 (8), 184 (100), 173 (12), 156 (34), 128 (58), 115 (12), 104 (23), 76 (35)

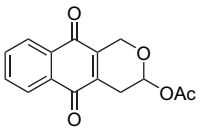


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Figure 3.

comparison of all the spectral data of the natural product only confirms our assumption that the product isolated from *Psychotria camponutans* is in fact psychorubrin (**2**) and not 1-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**3**) (Tables 2 and 3). Indeed, the cytotoxic activity of psychorubrin (**2**), which is also isolated from a *Psychotria* species, had already been established for long and is in accordance with the activity observed by Solis et al.[3]

Moreover, in order to substantiate their structural elucidation Solis et al. also prepared the acetyl derivative of the isolated natural product, for which the spectral data are also in accordance with analytical data of psychorubrin acetate (**13**) (Fig. 3).[2,3]

3. Conclusions

For the first time, a synthesis of 1-hydroxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**3**) was achieved based on a phthalide annulation reaction using 3-cyano-1(3*H*)-isobenzofuranone (**5**) and 5,6-dihydropyran-2-one (**6**) and subsequent reduction of the lactone moiety in the key steps. Combined with the synthesis of 3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**11**), different entries to the 3,4-dihydro-1*H*-isochromenedione skeleton have been disclosed, which is the basic skeleton for many pyranonaphthoquinone antibiotics. In contradiction with earlier reports in literature, psychorubrin (**2**) was identified to be the natural product isolated from *Psychotria camponutans*, to which significant antimalarial and cytotoxic activity have been allocated.

4. Experimental section

4.1. General experimental methods

Spectroscopic data were recorded as follows: ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra were recorded at 75 MHz. Peak assignments were performed with the aid of the DEPT technique, 2D-COSY, and HETCOR spectra. Mass spectra were recorded using a direct inlet system (70 eV) with a VL detector (ES, 4000 V). Elemental analyses were executed with a Perkin–Elmer Series II CHNS/O Analyzer 2400. The reported melting points are not corrected. Flash chromatography was carried out using a glass column with silica gel (particle size 0.035–0.07 mm, pore diameter ca. 6 nm). Solvent systems were determined via initial TLC analysis (Merck, silica gel 60F_{254}). Tetrahydrofuran, diethyl ether, and toluene were freshly distilled over sodium benzophenone ketyl.

4.2. Phthalide annulation reaction for the synthesis of 5,10-dimethoxy-3,4-dihydro-1H-benz[g]isochromen-1-one (4)

tert-Butanol (27.6 mmol), which was dried over CaH_2, was dissolved in dry THF (60 ml) and *n*-butyl lithium was added dropwise to the reaction mixture at 0 °C under nitrogen atmosphere, after which stirring was continued for 10 min. The reaction mixture was cooled to −60 °C and 3-cyano-1(3*H*)-isobenzofuranone (**5**) (25.1 mmol, 4.00 g), dissolved in THF (30 ml), was added dropwise to the reaction mixture. After 15 min of stirring at −60 °C, 5,6-dihydropyran-2-one (**6**) (25.1 mmol, 2.16 g), dissolved in THF (15 ml), was added dropwise. The resulting reaction mixture was stirred for 3 h at −60 °C and the temperature was allowed to warm to room temperature during a period of 14 h. The reaction mixture was quenched with hydrochloric acid (4 M) and solvent evaporation in vacuo resulted in the formation of 5,10-dihydroxy-3,4-dihydro-1*H*-benz[*g*]isochromen-1-one (**7**) as orange crystals, which were isolated by filtration. The aqueous phase was diluted with water and extracted with small portions of ethyl acetate in order to isolate the maximum amount of hydroquinone **7**. The combined organic extracts were washed with brine and dried over $MgSO_4$. Filtration and solvent evaporation in vacuo resulted in the isolation of 5,10-dihydroxy-3,4-dihydro-1*H*-benz[*g*]isochromen-1-one (**7**) as orange crystals.

Hydroquinone **7** was immediately protected as the corresponding methyl ether. Therefore, 5,10-dihydroxy-3,4-dihydro-1*H*-benz[*g*]isochromen-1-one (**7**) (24.3 mmol, 5.60 g) was dissolved in acetone (230 ml) to which potassium carbonate (121.5 mmol, 16.80 g) and dimethyl sulfate (53.5 mmol, 6.74 g) were added. The reaction mixture was heated under reflux for 2 h under nitrogen atmosphere. The mixture was allowed to cool to room temperature and was poured in water, followed by extraction of the aqueous phase with small portions of ethyl acetate. The combined organic extracts were washed with aqueous potassium hydroxide (4 M), brine and were dried ($MgSO_4$). Filtration and solvent evaporation in vacuo furnished 5,10-dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromen-1-one (**4**) as orange crystals.

4.2.1. 5,10-Dihydroxy-3,4-dihydro-1H-benz[g]isochromen-1-one (7)

^{1}H NMR ($CDCl_3$): δ 3.17 (2H, t, J=6.1 Hz, CH_2), 4.61 (2H, t, J=6.1 Hz, OCH_2), 7.52–7.71 (2H, m, H-7 and H-8), 8.01 (1H, d×d, J=0.6, 8.4 Hz, H-6), 8.42 (1H, d×d, J=0.6, 8.4 Hz, H-9), 11.96 (1H, s, OH). ^{13}C NMR (DMSO-d_6): δ 22.0 (CH_2), 68.2 (OCH_2), 101.8 (=C_{quat}), 115.7 (=C_{quat}), 122.1 (CH_{ar}), 123.1 (=C_{quat}), 123.2 (CH_{ar}), 125.7 (CH_{ar}), 129.4 (CH_{ar}), 130.2 (=C_{quat}), 139.5 (=C–O), 154.4 (=C–O), 170.6 (C=O). IR (KBr): ν_{max} 3348, 1639, 1223, 1176 cm^{-1}. MS (ES, neg. mode) *m/z* (%): 459 (2M−H^+, 90), 229 (M−H^+, 100), 227 (10), 113 (30).

4.2.2. 5,10-Dimethoxy-3,4-dihydro-1H-benz[g]isochromen-1-one (4)

Recrystallization from methanol yielded an analytical sample of **4** as pale orange needles, mp 149.0–149.5 °C. ^{1}H NMR

($CDCl_3$): δ 3.22 (2H, t, J=5.7 Hz, CH_2), 3.91 (3H, s, OCH_3), 4.10 (3H, s, OCH_3), 4.48 (2H, t, J=5.7 Hz, OCH_2), 7.57 (1H, d×d×d, J=0.9, 7.2, 8.3 Hz, H-7 or H-8), 7.67 (1H, d×d×d, J=0.9, 7.2, 8.3 Hz, H-7 or H-8), 8.10 (1H, d×d, J=0.6, 8.3 Hz, H-6), 8.33 (1H, d×d, J=0.6, 8.3 Hz, H-9). ^{13}C NMR ($CDCl_3$): δ 23.6 (CH_2), 61.8 (OCH_3), 63.4 (OCH_3), 66.8 (OCH_2), 113.8 (=C_{quat}), 121.9 and 124.5 (C-6 and C-9), 126.2 (=C_{quat}), 126.6 and 129.3 (C-7 and C-8), 128.9 (=C_{quat}), 131.2 (=C_{quat}), 147.4 (=C–O), 157.3 (=C–O), 162.5 (C=O). IR (KBr): ν_{max} 1716, 1621, 1208, 1152 cm^{-1}. MS (ES, pos. mode) *m/z* (%): 539 (2M+Na, 10), 259 (M+H$^+$, 100). Anal. Calcd for $C_{15}H_{14}O_4$: C 69.76, H 5.46. Found: C 69.59, H 5.60.

*4.3. Reaction of 5,10-dimethoxy-3,4-dihydro-1H-benz[g]-isochromen-1-one (**4**) with diisobutyl aluminum hydride (DiBAl-H)*

*4.3.1. Procedure for the synthesis of 5,10-dimethoxy-3,4-dihydro-1H-benz[g]isochromen-1-ol (**8**)*

5,10-Dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromen-1-one (**4**) (16.6 mmol, 4.30 g) was dissolved in toluene (220 ml) and DiBAl-H (19.92 mmol, 19.9 ml, 1.0 M in toluene) was added dropwise at −60 °C under nitrogen atmosphere. After 2.5 h the reaction mixture was quenched with hydrochloric acid (2 M) and the organic layer was separated. The aqueous solution was extracted once more with dichloromethane and the combined organic extracts were dried ($MgSO_4$). Filtration and solvent evaporation in vacuo resulted in the isolation of 5,10-dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromen-1-ol (**8**) as pale yellow crystals in 61% yield.

*4.3.2. 5,10-Dimethoxy-3,4-dihydro-1H-benz[g]isochromen-1-ol (**8**)*

Recrystallization from diethyl ether afforded **8** as pale yellow crystals, mp 140.4–140.7 °C. ^{1}H NMR ($CDCl_3$): δ 2.99–3.05 (2H, m, CH_2), 3.17 (1H, d, J=3.6 Hz, CH–O*H*), 3.89 (3H, s, OCH_3), 4.02–4.07 (1H, m, OCH_aH_b), 4.04 (3H, s, OCH_3), 4.34–4.43 (1H, m, OCH_aH_b), 6.39 (1H, d, J=3.6 Hz, C*H*–OH), 7.47–7.55 (2H, m, H-7 and H-8), 8.07 (2H, d, J=8.5 Hz, H-6 and H-9). ^{13}C NMR ($CDCl_3$): δ 22.9 (C-4), 57.3 (C-3), 61.2 (OCH_3), 63.2 (OCH_3), 88.5 (C-1), 122.3 and 122.7 (C-6 and C-9), 123.2 (=C_{quat}), 125.3 (=C_{quat}), 125.8 and 126.7 (C-7 and C-8), 127.4 (=C_{quat}), 128.7 (=C_{quat}), 149.4 (=C–O), 150.2 (=C–O). IR (KBr): ν_{max} 3338, 1592, 1455, 1444, 1428, 1354, 1271, 1113 cm^{-1}. MS (ES, neg. mode) *m/z* (%): 243 (M–OH$^-$, 100). Anal. Calcd for $C_{15}H_{16}O_4$: C 69.22, H 6.20. Found: C 69.06, H 6.31.

*4.3.3. Procedure for the synthesis of 5,10-dimethoxy-3,4-dihydro-1H-benz[g]isochromene (**10**)*

5,10-Dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromen-1-one (**4**) (16.6 mmol, 4.30 g) was dissolved in toluene (220 ml) and DiBAl-H (19.92 mmol, 19.9 ml, 1.0 M in toluene) was added dropwise at room temperature under nitrogen atmosphere. After 2 h the reaction mixture was quenched with hydrochloric acid (2 M) and the organic layer was separated. The aqueous solution was extracted once more with dichloromethane and the combined organic extracts were dried ($MgSO_4$). Filtration and solvent evaporation in vacuo resulted in the isolation of 5,10-dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromenene (**10**) as pale orange crystals in 43% yield.

*4.3.4. 5,10-Dimethoxy-3,4-dihydro-1H-benz[g]isochromene (**10**)*

Recrystallization from diethyl ether afforded **10** as pale orange crystals, mp 150.9–151.1 °C. ^{1}H NMR ($CDCl_3$): δ 3.14 (2H, t, J=5.7 Hz, CH_2), 3.90 (3H, s, OCH_3), 3.98 (2H, t, J=5.7 Hz, OCH_2), 4.00 (3H, s, OCH_3), 4.89 (2H, s, OCH_2), 7.47–7.55 (2H, m, H-7 and H-8), 8.01–8.05 (1H, m, H-6 or H-9), 8.08–8.12 (1H, m, H-6 or H-9). ^{13}C NMR ($CDCl_3$): δ 29.6 (C-4), 56.5 (C-1), 61.9 (OCH_3), 63.1 (C-3), 63.8 (OCH_3), 122.4 and 123.1 (C-6 and C-9), 126.1 and 126.6 (C-7 and C-8), 128.1 (=C_{quat}), 128.3 (=C_{quat}), 128.6 (=C_{quat}), 129.8 (=C_{quat}), 151.0 (=C–O), 151.5 (=C–O). IR (KBr): ν_{max} 1587, 1493, 1454, 1355, 1271, 1186 cm^{-1}. MS (ES, pos. mode) *m/z* (%): 245 (M+H$^+$, 100). Anal. Calcd for $C_{15}H_{16}O_3$: C 73.75, H 6.60. Found: C 73.68, H 6.53.

*4.4. Synthesis of 5,10-dimethoxy-3,4-dihydro-1H-benz[g]isochromene (**10**) by reduction of 5,10-dimethoxy-3,4-dihydro-1H-benz[g]isochromen-1-one (**4**) with lithium aluminum hydride ($LiAlH_4$)*

Lithium aluminum hydride (15.2 mmol, 0.58 g) was added at 0 °C under nitrogen atmosphere to 5,10-dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromen-1-one (**4**) (3.8 mmol, 1.00 g) in diethyl ether (50 ml). After 2 h of stirring at room temperature, the reaction mixture was quenched with hydrochloric acid (2 M) and filtered over Celite®. The organic phase was separated and the aqueous phase was extracted with small portions of diethyl ether. The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, and evaporated in vacuo. 5,10-Dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromenene (**10**) was isolated as pale orange crystals in 58% yield. For spectral data of 5,10-dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromene (**10**), see above.

*4.5. Cerium ammonium nitrate (CAN) mediated oxidative demethylation of 5,10-dimethoxy-3,4-dihydro-1H-benz[g]isochromen-1-ol (**8**) and 5,10-dimethoxy-3,4-dihydro-1H-benz[g]isochromene (**10**)*

General procedure: 5,10-Dimethoxy-3,4-dihydro-1*H*-benz[*g*]isochromenes **8** and **10** (2.69 mmol) were dissolved in acetonitrile (70 ml), and CAN (8.07 mmol, 4.42 g), dissolved in water (35 ml), was added dropwise at 0 °C, after which stirring was continued for 30 min at room temperature. The reaction mixture was poured in water and the aqueous phase was extracted with small portions of ethyl acetate. The combined organic extracts were washed with hydrochloric acid (2 M), dried ($MgSO_4$), filtered, and evaporated in vacuo.

*4.5.1. 1-Hydroxy-3,4-dihydro-1H-benz[g]isochromene-5,10-dione (**3**)*

Recrystallization from diethyl ether afforded **3** as a yellow powder with a purity of about 95% (^{1}H NMR). An analytical sample was prepared by preparative TLC (chloroform/ethyl acetate 9:1, R_f=0.22) and afforded **3** as yellow crystals, mp 215.7–216.1 °C. UV (MeOH) λ_{max} nm: 203, 246, 334. ^{1}H NMR ($CDCl_3$): δ 2.66–2.70 (2H, m, CH_2), 3.37 (1H, s, OH), 4.01–4.08 and 4.20–4.29 (2H, m, OCH_2), 6.05 (1H, s, C*H*–OH), 7.75–7.77 (2H, m, H-7 and H-8), 8.09–8.13 (2H, m, H-6 and H-9). ^{13}C NMR ($CDCl_3$): δ 22.1 (C-4), 56.6 (C-3), 85.8 (C-1), 126.5 (2×CH_{ar}), 131.8 (2×=C_{quat}), 133.9 (CH_{ar}), 134.1 (CH_{ar}), 140.5 (=C_{quat}), 143.6 (=C_{quat}), 183.3 (C=O), 184.3 (C=O). IR (KBr): ν_{max} 3413, 1658, 1635, 1592 cm^{-1}. MS (ES, neg. mode) *m/z* (%): 229 (M–H$^+$, 85), 227 (11), 211 (9), 201 (42), 199 (30), 186 (100), 185 (93), 173 (15), 171 (30), 158 (14), 159 (9). Anal. Calcd for $C_{13}H_{10}O_4$: C 67.82, H 4.38. Found: C 67.89, H 4.44.

*4.5.2. 3,4-Dihydro-1H-benz[g]isochromene-5,10-dione (**11**)*

Recrystallization from diethyl ether/chloroform 2:1 afforded **11** as pale yellow crystals, mp 132.8–134.5 °C. ^{1}H NMR ($CDCl_3$): δ 3.03 (2H, t, J=5.7 Hz, CH_2), 3.89 (2H, t, J=5.7 Hz, OCH_2), 4.70 (2H, s, OCH_2), 7.73–7.76 (2H, m, H-7 and H-8), 8.08–8.14 (2H, m, H-6 and H-9). ^{13}C NMR ($CDCl_3$): δ 29.6 (C-4), 56.0 (C-3), 60.9 (C-1), 126.5 and 126.5 (C-6 and C-9), 132.0 (=C_{quat}), 133.8 and 134.0 (C-7 and C-8), 145.2 (2×=C_{quat}), 184.9 (C=O), 185.5 (C=O). IR (KBr): ν_{max} 1666, 1621, 1595, 1328, 1294 cm^{-1}. MS (ES, pos. mode) *m/z* (%): 233 (M+H_2O, 100), 215 (M+H$^+$, 10). Anal. Calcd for $C_{13}H_{10}O_3$: C 72.89, H 4.71. Found: C 73.01, H 4.95.

*4.6. Synthesis of 1-methoxy-3,4-dihydro-1H-benz[g]isochromene-5,10-dione (**9**)*

1-Hydroxy-4,3-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**3**) (0.87 mmol, 0.20 g) was dissolved in dichloromethane (4 ml), after which silver(I) oxide (1.31 mmol, 0.30 g) and iodomethane (1.74 mmol, 0.25 g) were added. The reaction mixture was protected from light and was stirred in a closed recipient under nitrogen atmosphere for 24 h. Filtration over Celite® and washing with dichloromethane followed by solvent evaporation in vacuo furnished 1-methoxy-3,4-dihydro-1*H*-benz[*g*]isochromene-5,10-dione (**9**), which was recrystallized from methanol.

*4.6.1. 1-Methoxy-3,4-dihydro-1H-benz[g]isochromene-5,10-dione (**9**)*

Recrystallization from methanol afforded **9** as yellow crystals, mp 151.0–151.2 °C. ^{1}H NMR ($CDCl_3$): δ 2.65 (2H, t, J=5.6 Hz, CH_2), 3.59 (3H, s, OCH_3), 3.94–4.01 and 4.06–4.15 (2H, m, OCH_2), 5.51 (1H, s, C*H*–OCH_3), 7.69–7.76 (2H, m, H-7 and H-8), 8.06–8.10 (2H, m, H-6 and H-9). ^{13}C NMR ($CDCl_3$): δ 22.0 (C-4), 56.1 (C-3), 56.3 (OCH_3), 92.3 (C-1), 126.3 and 126.4 (C-6 and C-9), 131.8 (=C_{quat}), 131.9 (=C_{quat}), 133.7 and 134.0 (C-7 and C-8), 139.7 (=C_{quat}), 143.3 (=C_{quat}), 182.6 (C=O), 184.5 (C=O). IR (KBr): ν_{max} 1666, 1636, 1594, 1329, 1295 cm^{-1}. MS (ES, neg. mode) *m/z* (%): 213 (M–MeO$^-$, 100). Anal. Calcd for $C_{14}H_{12}O_4$: C 68.85, H 4.95. Found: C 69.03, H 5.00.

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